

## Adding value to rare tissue samples donated to biobanks: characterisation of breast tissue and primary cell cultures obtained from a female-to-male transgender patient

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**Abstract** Biobanks provide a window of opportunity to store and add value to material from rare cases allowing their future use in biomedical research. One such example is the opportunity to obtain good quality tissue from patients undergoing gender re-assignment. Following patient agreement to donate tissue samples

to our biobank we catalogued the histological appearance, defined the expression of the hormone receptors ER $\alpha$ , PR, AR and the proliferation marker Ki67, and generated and characterised primary cell cultures in a female to male (FTM) transgender patient referred to our unit for surgery. Immunohistochemistry was performed for ER $\alpha$ , PR and AR and the proliferation marker Ki67. Hormone receptor expression was confined to epithelial cells lining the breast ducts. Ki67 immunoreactivity was sparse indicating little proliferation of luminal epithelium, consistent with normal mammary gland. Cultures of epithelial cells and fibroblasts were derived from surplus tissue. The latter lacked expression of epithelial markers and hormone receptors but exhibited expression of vimentin. Culture of the former on Matrigel saw an outgrowth of more rounded “epithelial-like” cells. Immunofluorescence characterisation showed a mixed phenotype with expression of vimentin and both myoepithelial and luminal epithelial markers. Sporadic weak ER $\alpha$  expression and moderate PR expression was seen. In summary, as well as routinely collecting tissue and blood samples, we have characterised and stored tissue and cells from a FTM transgender patient, adding value to this resource which, available from the Breast Cancer Campaign Tissue Bank for those interested in further studying the biology of FTM transgender tissue.

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## Introduction

Biobanking is now becoming embedded in biomedical research, providing opportunities for scientists to study human tissues as adjuncts to established cell lines. The Leeds Breast Unit is a centre for breast tissue biobanking in the UK and is a founding member of the Breast Cancer Campaign Tissue Bank (<http://www.breastcancercampaigntissuebank.org>). As an approved Research Tissue Bank, we approach all patients who present to our breast clinic to consent for the storage and use of their surplus tissue and additional blood samples to use in biomedical research. As expected the vast majority of the tissue obtained is from female patients undergoing surgery for breast cancer or for a cosmetic breast reduction. Tissue is also obtained from consenting patients with male breast cancer, which is much rarer.

Gender re-assignment is a complex multimodality process involving extensive counselling, surgical procedures and medical interventions, which are dominated by the manipulation of hormones. For female-to-male (FTM) gender change, this involves administration of testosterone either in the form of intramuscular injections or the application of gels or patches while in younger patients GnRH analogues are often administered in order to delay puberty until the patient is ready to make a decision to commence hormone manipulation (Spack 2013). Since it is well established that lifetime oestradiol exposure plays a part in the pathogenesis of breast cancer, examination of breast tissue removed in circumstances where exposure to hormones is either reduced or increased could provide some valuable clues on the influence of these hormones on the biology and morphology of breast tissue. However, opportunities to obtain good quality tissue and primary cells from patients undergoing gender re-assignment are extremely rare.

The aim of this study was to catalogue the histological appearances and define the expression of the hormone receptors ER $\alpha$ , PR, AR and the proliferation marker Ki67 in a FTM transgender patient who was referred to our unit for surgery in 2012 and who consented for their tissue to be stored in a national biobank. As a means of adding value to this case, we generated and characterised primary epithelial and fibroblast cell cultures from this patients samples. These samples are available to researchers to study broader issues of mammary gland biology.

## Methods

### Patient information and surgical procedure

A 24 year Caucasian patient, born female, commenced gender reassignment 8 years earlier. For the previous 6 years they received three-monthly intramuscular Testosterone (250 mg). In November 2012 the patient underwent a bilateral mastectomy through elliptical skin incisions. This included both nipple areola regions in view of a number of on-going medical issues, the surgical decision after extensive discussions with the patient and their medical team was to limit surgery and not offer any nipple-areolar reconstruction which would necessitate future further operations.

### Blood samples

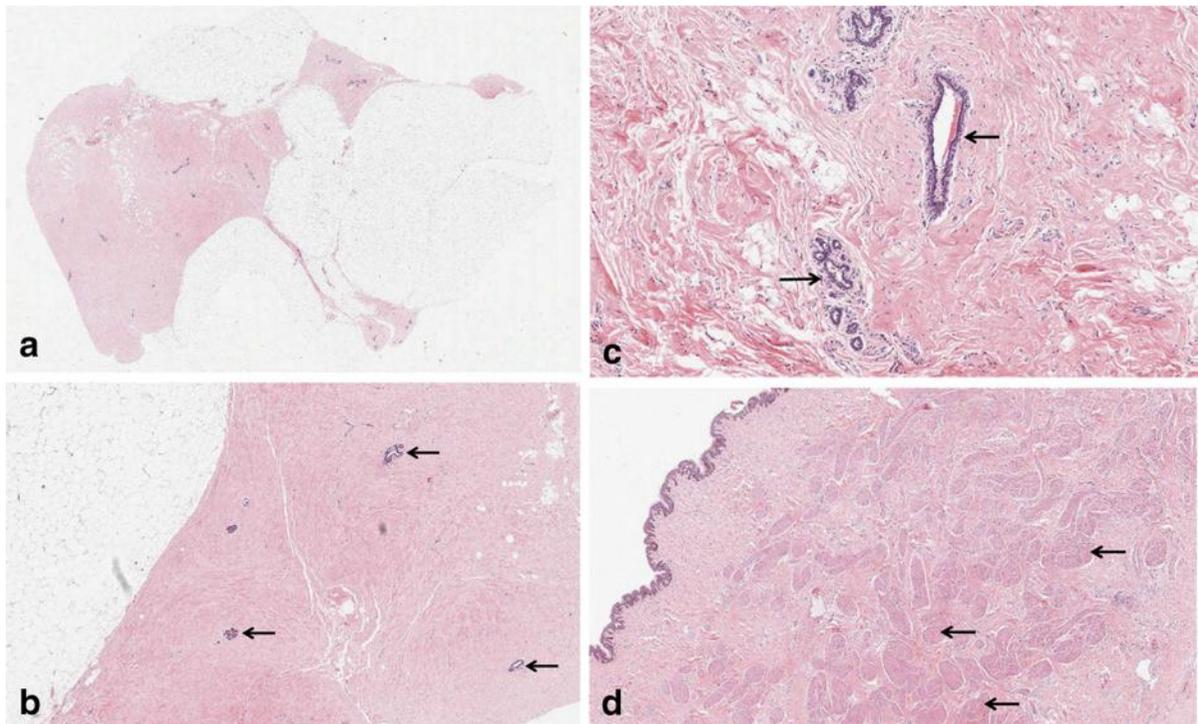
Blood samples were collected from the median cubital vein first into a red-topped plain glass blood tube (serum) followed immediately by a lavender-topped tube containing EDTA anticoagulant (plasma). Whole blood was snap frozen. The serum sample was allowed to clot at room temperature for 30 min. Thereafter serum and plasma was isolated from each blood tube by whole blood centrifugation (850 g, 10 min, room temperature). All fluid samples were aliquoted and frozen at  $-80^{\circ}\text{C}$ .

### Tissue procurement, primary cell culture and characterisation

Following written informed consent (09/H1306/108; Leeds (East) Research Ethics Committee), surplus tissue was obtained from histopathology. This was either immediately snap frozen in liquid nitrogen or processed for primary cell culture. Cell cultures were generated according to a previously published method (Holliday et al. 2009) except that organoids were cultured in Keratinocyte Serum Free Media (Speirs et al. 1998). All cells were incubated at  $37^{\circ}\text{C}$  and 5 %  $\text{CO}_2$ . Epithelial cells and fibroblasts were cultured on glass coverslips and characterised as previously (Holliday et al. 2009). Images were recorded with a Nikon A1 confocal microscope.

### Immunohistochemistry

Formalin-fixed paraffin-embedded tissue sections were prepared following routine histology procedures



**Fig. 1** Digital H&E images of a section of the FTM breast tissue in which the vaguely nodular appearance of the adipose tissue can be appreciated (**a**,  $\times 0.3$ ); an area of fibrous tissue containing scattered breast glandular elements (*arrows*)

consisting mainly of ductal structures (**b**,  $\times 2$ ); a residual lobule which appears atrophic containing only a couple of acini (*arrows*), magnification (**c**,  $\times 6.9$ ); section of nipple showing prominent smooth muscle bundles (*arrows*; **d**,  $\times 2$ )

and stained for ER $\alpha$ , PR, AR and Ki67 according to previously published protocols (Murphy et al. 2006).

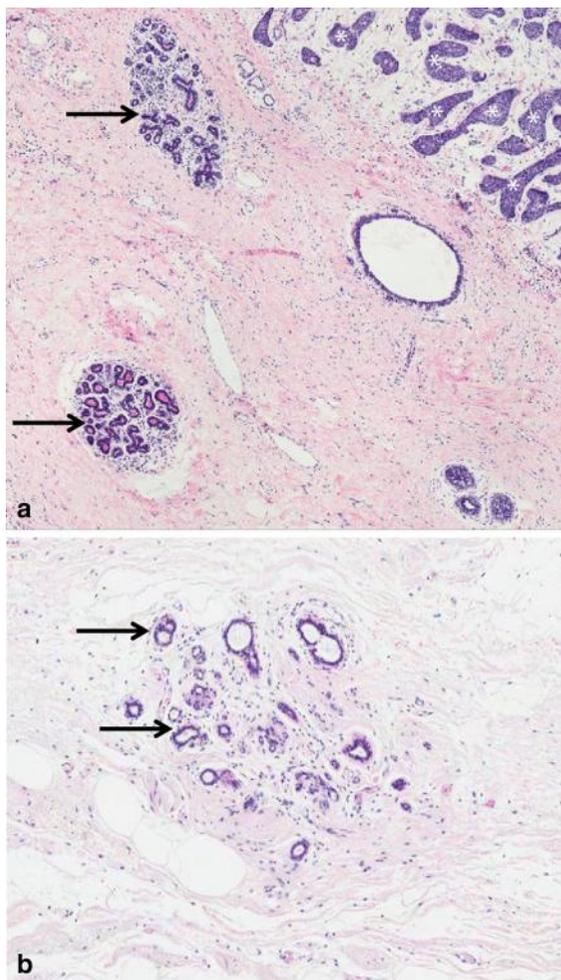
## Results

Surgery was uneventful with no post operative complications and good wound healing. On macroscopic examination, it was noted that the skin overlying the breast was hair-bearing. The left breast weighed 830 g and the right breast 845 g. The cut surface of the breast tissue consisted of vaguely nodular areas of adipose tissue interspersed with soft fibrous tissue. No discrete lesions were identified. On microscopic examination, the nodular appearance of the tissue was also apparent (Fig. 1a). The fibrous areas contained scattered breast glandular elements consisting mainly of ductal structures with only occasional lobules (Fig. 1b). The sparse lobules that were present appeared atrophic with a reduced number of acini (Fig. 1c) Sections from the nipple showed prominent smooth muscle bundles (Fig. 1d).

As obtaining completely normal male breast tissue is challenging, by way of comparison, the morphology of a typical case of male breast carcinoma which contained tissue adjacent normal tissue available from our local archive is shown in Fig. 2a. Here, scattered islands of breast lobules were observed. Tissue from a postmenopausal female reduction mammoplasty is additionally shown (Fig. 2b) and also contains breast lobules. These images confirm the difference in appearance of the transgender tissue from normal male and female breast tissue.

Immunohistochemistry was performed for three hormone receptors (ER $\alpha$ , PR, AR) and the proliferation marker Ki67. Serial sections in Fig. 3 show staining was confined to epithelial cells lining the breast ducts. Ki67 immunoreactivity was sparse indicating little proliferation of the luminal epithelial cell, consistent with normal mammary gland.

Cultures of epithelial cells and fibroblasts were derived from surplus tissue. The fibroblast enriched population (Fig. 4a) lacked expression of epithelial markers and hormone receptors but exhibited expression



**Fig. 2** Low power ( $\times 4$ ) digital H&E images of normal male breast tissue adjacent to breast carcinoma (**a**) and postmenopausal female breast tissue (**b**). The presence of breast ducts and lobules is shown (*arrows*). *Asterisks* in **a** indicates carcinoma cells in the top right area of the image

of the mesenchymal marker vimentin. Culture of small fragments of fibroblast depleted tissue on Matrigel (Fig. 4b) saw an outgrowth of more rounded “epithelial-like” cells. Immunofluorescence characterisation showed this cell population had a mixed phenotype that expressed the mesenchymal marker vimentin and both myoepithelial and luminal epithelial markers. Sporadic weak ER $\alpha$  expression and moderate PR expression was seen in these in vitro cell cultures. Frozen cell culture aliquots are available in the Breast Cancer Campaign Tissue Bank (<http://www.breastcancercampaigntissuebank.org>), either as viable tissue explants, partly

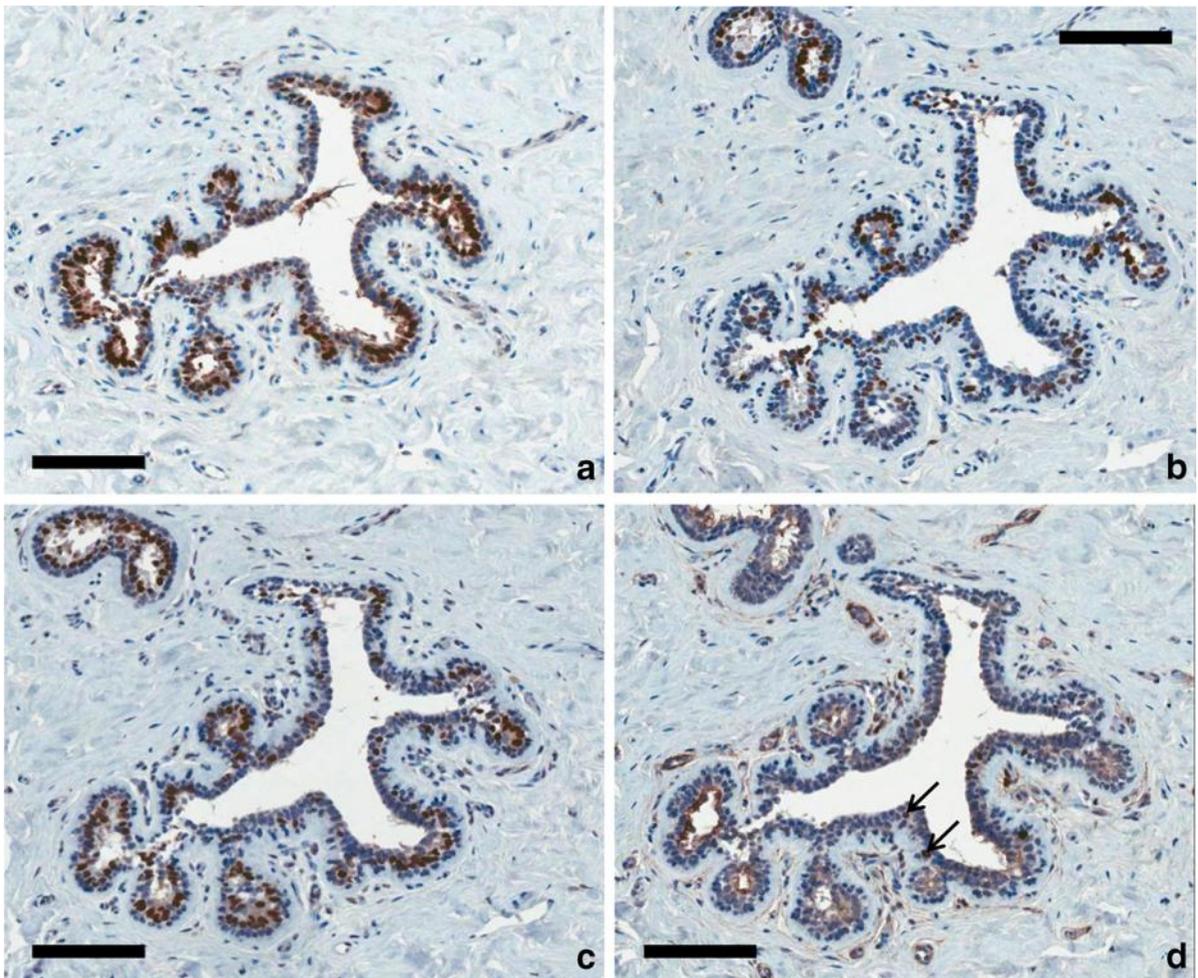
purified organoid preparations and as epithelial or fibroblast-enriched populations.

In addition, 28 aliquots of tissue (approximately  $5 \times 5 \times 5$  mm) were snap frozen in liquid nitrogen then immediately transferred to  $-80$  °C for long term storage including mirror banking on separate electrical supplies. A further 50 formalin-fixed paraffin embedded tissue blocks were prepared. One whole blood, one plasma and two serum samples were processed and stored.

## Discussion

Opportunities to obtain tissue from FTM transgender patients are limited. As a result there is inadequate understanding of how long term hormone treatment may affect the morphology and histology of breast tissues. In 2012 a 24 year old FTM transgender was referred to the Leeds Teaching Hospitals NHS Trust for surgery. Surgery was considered risky as the patient had a congenital heart condition but was completed with no adverse events. The patient agreed to consent for their tissue to be used in research and has been deposited in a specialist national biobank, making this resource more widely available to the biomedical research community.

The early stages of mammary development are independent of sex steroid hormones but at the 15th week of fetal development, the breast tissue is transiently sensitive to testosterone (Sternberg 1992). If significant testosterone exposure occurs, the development of the alveolar ductal system is prevented; if not, the milk ducts are formed by weeks 20–32. At puberty, the effect of oestrogen, progesterone and growth hormone results in further breast growth and development in females. In males, there is commonly some benign enlargement of the male breast (gynaecomastia) at the time of puberty due to a temporary imbalance between oestrogen and testosterone with relatively more oestrogen being produced (Braunstein 2007). The gynaecomastia usually regresses spontaneously as the balance shifts towards higher testosterone and lower oestrogen levels. As long-term testosterone treatment is a requirement for FTM gender reassignment we wished to explore the effects this may have on fully developed female breast tissue; while female sex steroids are recognised to have profound effects on female breast tissue, and influence breast



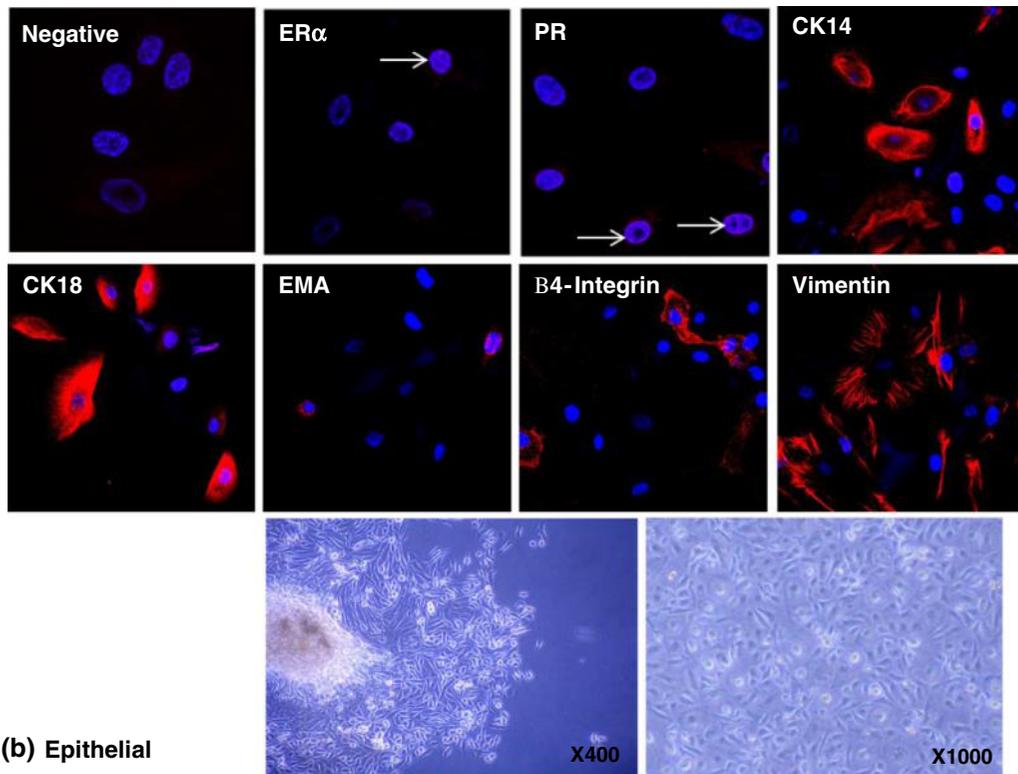
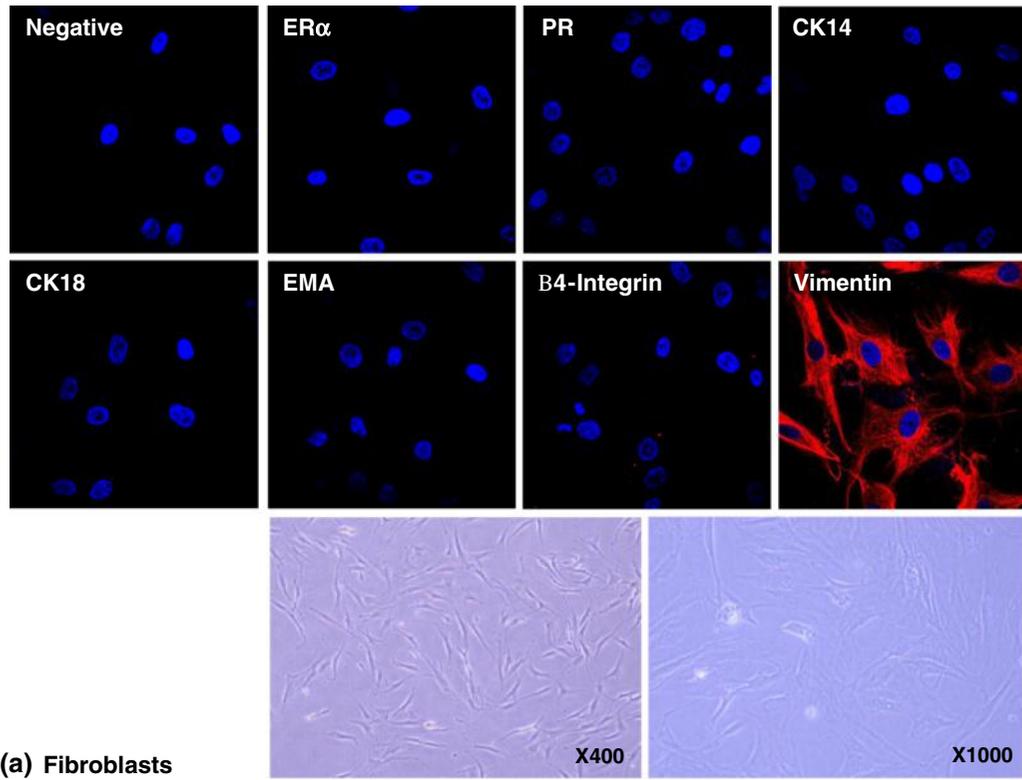
**Fig. 3** Immunohistochemistry of ER $\alpha$  (a), PR (b), AR (c), Ki67 (d) in semi serial sections of FTM breast tissue. All 3 hormone receptors are expressed but Ki67 expression is sparse (arrows). Scale bar 100  $\mu$ m

development, differentiation and growth, the impact of sustained elevated levels of testosterone is less clear.

Histological examination in this case revealed diffuse regression of well-formed lobules. The appearance was reminiscent of what is sometimes seen in the post-menopausal female breast, as previously reported (Slagter et al. 2006). This is perhaps unsurprising given that the change in oestrogen/testosterone balance is similar, though obviously to lesser degrees. We also noted that the smooth muscle bundles in the area of the nipple were particularly prominent more so than what is usually seen in sections from both female and male nipples. The significance of this, and whether it relates to the effect of testosterone, is uncertain, particularly as other studies have shown no effects of

prolonged testosterone exposure on breast histology or immunohistochemical profiles (Burgess and Shousha 1993). Histologically this differed from normal male breast tissue adjacent to carcinoma and of female breast tissues from a cosmetic reduction mammaplasty. While it could be argued that neither of these can be considered completely normal, sourcing completely normal male breast tissue and to a lesser extent female breast tissue can be challenging, with adjacent normal material often used by researchers as a comparator.

Immunohistochemical examination of ER $\alpha$ , PR, AR and Ki67 showed the presence of all 3 hormone receptors in the luminal epithelium lining the ducts. All 3 steroid hormone receptors seemed to be



◀ **Fig. 4** Morphological appearance of primary fibroblast (a) and epithelial (b) cell cultures derived from the FTM transgender and their characterisation by immunofluorescence using specific markers. In **b** arrows refer to weak nuclear ER $\alpha$  or PR expression

represented at roughly even levels with coexpression in some cells. Ki67 expression was sparse with occasional solitary cells expressing this biomarker. This is consistent with normal mammary gland. A gene expression study profiled breast biopsies from five FTM transgender patients taken before and after 2 years of testosterone. The study showed upregulation of 243 and downregulation of 2007 genes. Interestingly those which were upregulated were associated with breast cancer-related expression signatures including *Jun* and *Fos*. Histological examination of a further FTM transgender series revealed a non-uniform appearance of the tissues, although one of the five cases had regression in the glandular tissue similar to our observations (Bentz et al. 2010). Other common features were fibroadenomatous hyperplasia and apocrine metaplasia, neither of which was observed in our case. ER $\alpha$  was present in all cases with PR expressed in 2/5.

As a means of adding value to this rare case, we successfully isolated stromal and epithelial cell populations and characterised their morphology. To our knowledge this has not been done previously in FTM transgender patients. Upon immunofluorescence characterisation, the former lacked expression of epithelial markers and hormone receptors but exhibited strong expression of the mesenchymal marker vimentin. This immunoprofile reflects a mesenchymal fibroblast phenotype which is further supported by their characteristic spindle-shaped appearance in culture. Culture of small fragments of fibroblast depleted tissue on Matrigel<sup>TM</sup> saw an outgrowth of more rounded “epithelial-like” cells. Immunofluorescence characterisation showed this cell population to have a mixed phenotype that expressed the mesenchymal marker vimentin and both myoepithelial and luminal epithelial markers. This cell population weakly expressed ER $\alpha$  upon in vitro culture and moderately expressed PR. This mixed immunoprofile could suggest the presence of an epithelial progenitor cell type which under further processing and specific culture conditions may have potential to differentiate into specific luminal or myoepithelial cell types; there is scope to

further sort the epithelial cell population into luminal and myoepithelial cells.

In summary we have generated, characterised and banked tissue and cells from a FTM transgender patient. This is a valuable resource for those interested in further studying the biology of FTM transgender tissue or broader issues around mammary gland biology. To our knowledge the primary cell cultures we have generated from this case are unique. Multiple aliquots of frozen and formalin-fixed paraffin embedded tissue samples and cell culture aliquots described have been deposited in the Breast Cancer Campaign Tissue Bank. Nucleic acids can be extracted and provide to researchers if required and germline DNA is available through whole blood. Information on how to apply for these samples can be found on the web site (<http://www.breastcancercampaigntissuebank.org>).

Finally, in the absence of a structured biobanking system, it may not always be possible to collect rare tissues such as the case described. While rare tissue types do exist in histopathology archives they are often a hidden resource and generally only available as formalin-fixed tissue blocks which, even with the range of modern molecular biology technologies available nowadays, may limit their use. This emphasises the value of biobanks which offer valued added materials over and above their standard frozen/FFPE portfolio, providing researchers with additional experimental tools to help understand disease processes.

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**Conflict of interest** None declared.

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